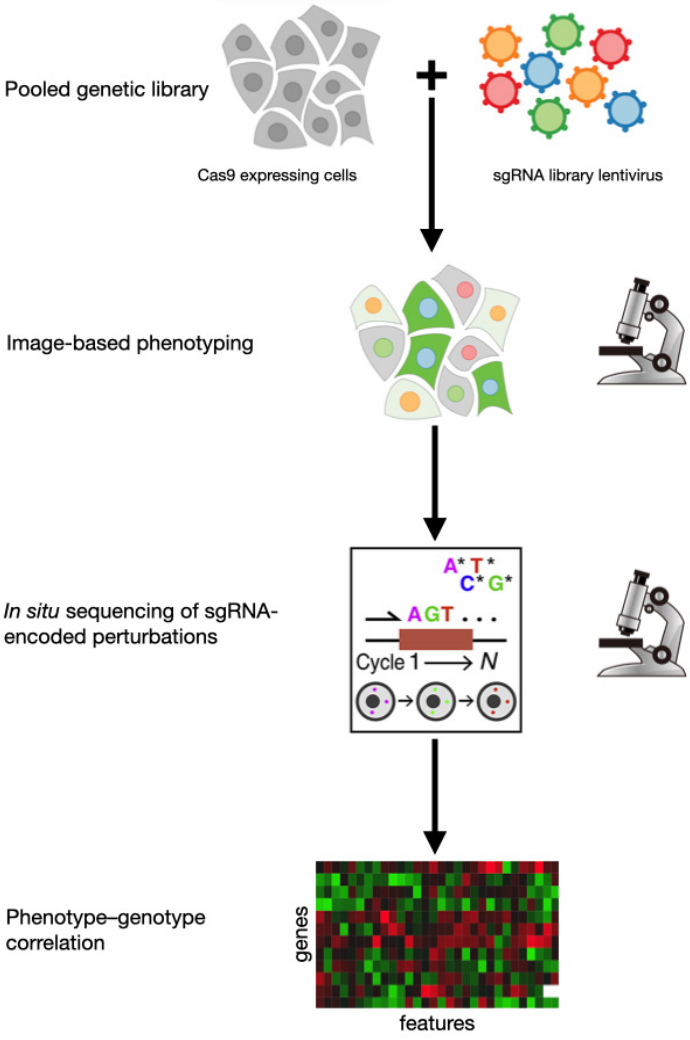
Optical Pooled Screening using the CELESTA Light Engine

The advent of CRISPR gene editing technology has created the need for large-scale characterization of phenotypes resulting from manipulated genotypes. Optical pooled screening (OPS) is one of several genetic screening techniques developed for this purpose [1]. In an optical pooled screen, a genetically diverse library of living cells is imaged and characterized for phenotypic variations without knowing the genotype of the cells. The genotypes are identified *in situ* after the cells have been fixed or by physical extraction of interesting phenotypes, followed by sequencing (Figure 1). Much of the development of OPS has been carried out in the laboratory of Professor Paul Blainey at Massachusetts Institute of Technology [2]. The CELESTA Light Engine is the laser illuminator of choice for both immunofluorescence phenotyping and *in situ* sequencing. The high power light output of the CELESTA allows exposure times for *in situ* sequencing to be cut by a factor of 5—10 compared to earlier protocol implementations using a SOLA Light Engine. Since *in situ* sequencing requires multiple cycles of dye incorporation, imaging and cleavage, the overall time saving is substantial. Recently, Blainey and co-workers expanded the capabilities of OPS to allow analysis of 80,000 CRISPR knockout perturbations from single-cell images of about 10 million cells [3]. This provided new insights into previously unresolved changes in the subcellular localization of host and viral proteins during Sendai virus infection of mammalian cells.

Such demanding OPS techniques leverage CELESTA's optical power, spectral purity and breadth, and stability, depending on robust laser illumination for speed, diverse multiplexing, and consistency.



References

[1] Pooled genetic screens with image-based profiling. RT Walton, A Singh, PC Blainey (2022) Mol Syst Biol 18:e10768

[2] Pooled genetic perturbation screens with image-based phenotypes. D Feldman, L Funk PC Blainey et al. (2022) Nat Protoc 17:476–512

[3] A genome-wide optical pooled screen reveals regulators of cellular antiviral responses. RJ Carlson, MD Leiken, PC Blainey et al. (2023) Proc Natl Acad Sci U S A 120:e2210623120